

PCT

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(3)

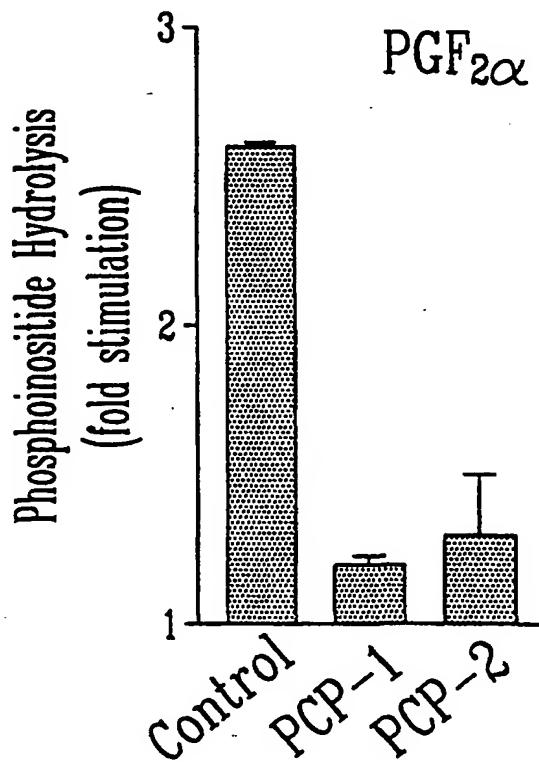
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(54) Title: ANTAGONISTS OF G-PROTEIN-COUPLED RECEPTOR

(57) Abstract

The present invention relates to a new class of G-protein-coupled receptor antagonists which bind to the intracellular molecular interface between the receptor and the G-protein, thus hampering signal transduction. The present invention describes peptide sequences derived from the prostaglandin receptor F_{2α} and the G-protein, G_{αq} protein, produced by molecular biology techniques or chemical synthesis, as selective inhibitors of signal transduction involved in the stimulation of this receptor. Such peptides or molecules derived from their primary, secondary and tertiary structures may be used as effective tocolytics for the prevention of premature labor or be utilized for the treatment of dysmenorrhea.



ANTAGONISTS OF G-PROTEIN-COUPLED RECEPTORBACKGROUND OF THE INVENTION(a) Field of the Invention

5 The invention relates to G-protein-coupled receptor antagonists which bind to the intracellular molecular interface between the receptor for PGF_{2α} (FP receptor) and the G-protein.

(b) Description of Prior Art

10 Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomerular filtration, 15 mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation. Prostanoids mediate their actions through binding to distinct 20 receptors which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins consisting of α, β and γ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of 25 cyclic adenosine monophosphate synthesis (Strader, C.D., et al., Ann. Rev. Biochem. 63: 101-132, 1994).

Of the five pharmacologically distinct prostanoid receptors for E₂, I₂, D₂, TXA₂ and F_{2α} and their 30 many isoforms, the receptor for PGF_{2α}, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine myometrium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is 35 marked by tremendous rise in PGF_{2α} levels and increased

uterine contractility. The wide spread use of PGF_{2α} analogues to induce labor in veterinary industry points to the primary role of PGF_{2α} and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto, Y., et al., *Science*, 277: 81-83, 1997).

In face of escalating costs incurred as a result of premature births and associated complications to the neonate, such as intraventricular hemorrhage, bronchopulmonary dysplasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature labor is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly PGE₂ and PGF_{2α}. However, inhibition of the former is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension. At another level, PGF_{2α} has been attributed a major role in dysmenorrhea, a condition which afflicts 5%-7% of premenopausal women. A pre-menstrual increase in PGF_{2α} levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended therapy hampered the advances in preventing premature labor and associated sequelae.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares only 47% sequence identity with EP1 receptor, and to a lesser extent with other prostanoid receptors (Abramovitz, M., et al., *J. Biol. Chem.*, 269: 2632-2636, 1994). Binding of PGF_{2α} to FP receptor is followed by the activation of G_{αq/βγ} complex, increased GTP binding by the G_{αq} subunit, stimulation of phospholi-

pase β activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction. The FP receptor is the only efficacious target for development of therapeutic drugs since a few G_{α} -proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors; their efficacy will be compromised in face of tremendous increase in PGF_{2 α} concentrations in myometrium at the onset of labor; and the basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

It would be highly desirable to be provided with antagonists of FP receptors which do not cross-react with other prostanoid receptors and which are effective even in the absence of a ligand.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide antagonists of FP receptors which do not cross-react with other prostanoid receptors and which are effective even in the absence of a ligand.

Another aim of the present invention is to provide inhibitors of FP receptors devised by a novel strategy to target the intracellular surface of the receptor at which the cytoplasmic domains of the FP receptor and the G_{α} protein interact.

In accordance with the present invention there is provided a receptor prostanoid receptor antagonist which binds to an intracellular molecular interface

between a receptor and a G_{α} -protein, wherein said antagonist has a first amino acid sequence coding for a third or fourth intracellular domain, or a part thereof, and a second amino acid sequence coding for 5 α -helices of a G_{α} protein, whereby when bound to the intracellular molecular interface, said antagonist hampers signal transduction from said receptor.

The receptor is preferably the PGF_{2 α} receptor of prostaglandin.

10 The antagonist of the present invention preferably comprises an amino acid sequence derived from the sequence of at least one of the prostaglandin F_{2 α} receptor and the associated protein $G_{\alpha q}$. More preferably, the antagonist of the present invention consist in 15 an amino acid sequence of the FP receptor selected from the group consisting of RVKFKSQQHR QGRSHHLEM (SEQ ID NO:3) and RKAVALKNLYK LASQCCGVHV ISLHIWELSS IKNSLKVAII SESPVAEKSA ST (SEQ ID NO:4).

In accordance with the present invention there 20 is also provided a method for preventing premature delivery of fetus comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G-protein-coupled receptor antagonist which binds to an intracellular molecular 25 interface between a receptor and a G-protein, wherein the antagonist, when bound to the intracellular molecular interface, hampers the transduction of a signal, thereby reducing contractions.

In accordance with the present invention there 30 is also provided a method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G-protein-coupled receptor antagonist which binds to an intracellular molecular 35 interface between a receptor and a G-protein, wherein

the antagonist, when bound to the intracellular molecular interface, hampers the transduction of a signal thereby reducing pain associated with contractions.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B illustrate the inhibitory effects of PCP-1 and PCP-2 on FP receptor function upon stimulation with PGF_{2α} or PGE₂, in accordance with one embodiment of the present invention;

10 Fig. 2A illustrates the effects of G_{αq}-derived peptides on FP receptor function; and

Fig. 2B illustrates a dose-response of PCP-4 on PGF_{2α} receptor function.

15 DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a new class of G-protein-coupled receptor antagonists which bind to the intracellular molecular interface between the receptor and the G-protein, thus hampering signal transduction.

Hence, a novel strategy to target the intracellular surface of the receptor at which the cytoplasmic domains of FP receptor and Gq protein interact was designed. By preventing the binding of Gq protein to 25 FP receptor with the inhibitors of the present invention, derived from both the FP receptor and Gq protein, FP receptor function in the presence of its ligand was diminished. Furthermore, the specificity of the 30 inhibitors of the present invention is demonstrated by analyzing the function of a highly related prostaglandin receptor, EP1.

PREPARATION OF INHIBITORS

Cell Culture

A549 lung carcinoma cells (ATCC Accession No.: 35 CCL185, American Type Culture Collection, Rockville, MD

20852) were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics, penicillin (10 U/ml) and streptomycin (10 µg/ml) in a humidified atmosphere containing 5% CO₂ at 5 37°C. The cells were trypsinized and plated in 6-well tissue culture dishes at 2 X 10⁵ cells/well, a day before commencing the experiments.

Cloning FP receptor intracellular domains

The DNA fragments 5' AGA GTT AAA TTT AAA AGT 10 CAG CAG CAC AGA CAA GGC AGA TCT CAT CAT TTG GAA ATG 3' (SEQ ID NO:1) and 5' CGA AAG GCT GTC CTT AAG AAT CTC TAT AAG CTT GCC AGT CAA TGC TGT GGA GTG CAT GTC ATC AGC 15 TTA CAT ATT TGG GAG CTT AGT TCC ATT AAA AAT TCC TTA AAG GTT GCT GCT ATT TCT GAG TCA CCA GTT GCA GAG AAA TCA GCA AGC ACC 3' (SEQ ID NO:2), encoding the intracellular domains of the FP receptor having the following amino acid sequences: RVKFKSQQHR QGRSHHLEM (SEQ ID NO:3) (PCP-1) and RKAVALKNLYK LASQCCGVHV ISLHIWELSS IKNSLKVAII SESPVAEKSA ST (SEQ ID NO:4) (PCP-2) were cloned by RT- 20 PCR. Total mRNA from human foreskin fibroblasts were prepared by acid phenol-guanidine isothiocyanate method (Chomczynski, P., and Sacchi, N., *Anal. Biochem.*, **162**: 156-159, 1987).

Reverse transcription followed by amplification 25 of the cDNAs using the gene-specific primers pcp 1.1: 5' GCG TCT AGA ATG AGA GTT AAA TTT AAA AGT CAG 3' (SEQ ID NO:5), pcp 1.2: 5' GCG TCT AGA CTA CAT TTC CAA ATG ATG 3' (SEQ ID NO:6) pcp 2.1: 5' CGC TCT AGA ATG CGA AAG GCT GTC CTT AAG 3' (SEQ ID NO:7) and pcp 2.2: 5' 30 GCG TCT GAG CTA GGT GCT TGC TGA TTT CTC 3' (SEQ ID NO:8), derived from the human FP receptor sequence (Abramovitz, M., et al., *J. Biol. Chem.*, **269**: 2632-2636, 1994) and Taq™ polymerase (GIBCO Life Technologies, Burlington, ON) were conducted as described by 35 Peri et al. (Peri, K.G., et al., *J. Biol. Chem.*, **270**:

24615-24620, 1995). Briefly, two micrograms of total RNA was reverse transcribed using 400 U of M-MLV reverse transcriptase and 10 µg/ml random hexamers, in a 50 µl reaction containing 50 mM Tris-HCl, pH 8.3, 75 mM KC1, 3 mM MgCl₂, 10 mM DTT, and 0.5 mM each of dCTP, dGTP, dATP and dTTP, for 1 h, at 42°C. An aliquot of the cDNA (equivalent to 1 µg of RNA) was amplified using 1.5 U Taq DNA polymerase in a 100 µl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KC1, 1.5 mM MgCl₂, 0.2 mM each of dCTP, dGTP, dATP and dTTP, and 0.5 µM each of the primers, for 35 cycles (each cycle was 94°C, 1 min.; 50°C, 1 min.; and 72°C, 1 min.).

The PCR products were digested with Xba I restriction enzyme (GIBCO Life Technologies, Burlington, ON) and cloned into the Xba I site of pRC-CMV vector (Invitrogen, CA). Multiple plasmid clones were sequenced using the T7 sequencing kit (Pharmacia, Baie D'Urfe, PQ) to verify the sequence of the cDNAs.

Cell transfection and selection of G418-resistant clones

The expression plasmids carrying the third (PCP-1) and the fourth (PCP-2) intracellular domains were introduced into A549 cells using Transfectamine lipid (GIBCO Life Technologies, Burlington, ON). Three (3) micrograms of DNA and 16 µg of lipid were mixed in 200 µl of water and incubated at room temperature for 45 min. Then, the lipid-DNA complexes were diluted with 0.8 ml of Opti-MEM™ (GIBCO Life Technologies, Burlington, ON). The cells were washed twice with Hank's Balanced Salt Solution and incubated with lipid-DNA complexes for 6 hours. An equal volume of DMEM with 20% FBS was added and the cells were kept in the incubator overnight. On the next day, the medium was

replaced with DMEM containing 10% FBS and antibiotics and incubated for another 24 hours.

On the following day, the cells were trypsinized and plated in 100 mm cell culture dishes at 1 x 5 10^4 cells/ml in DMEM containing 10% FBS, antibiotics and 1 mg/ml of G418 (GIBCO Life Technologies, Burlington, ON). The G418 containing medium was replaced every 3 days. G418-resistant colonies were trypsinized and pooled for further analysis. The expression of 10 PCP-1 and PCP-2 peptides was tested by analyzing mRNA expression using RNase protection assays as described by Peri et al. (Peri, K.G., et al., *J. Biol. Chem.*, 270: 24615-24620, 1995). More particularly, total RNA 15 was isolated using acid phenol guanidine isothiocyanate method (Chomczynski, P., and Sacchi, N., *Anal. Biochem.*, 162: 156-159, 1987). Aliquots of total RNA (10 μ g) were mixed with 5 x 10^5 cpm of [32 P]-labeled cRNA probes (synthesized from pIL3 and pIL4 plasmids which 20 are expression plasmids encoding PCP-1 and PCP-2 peptides, using a commercial *in vitro* transcription kit sold by Promega, Madison, WI) in a solution containing 80% (v/v) formamide, 40 mM PIPES, pH 6.8 and 0.4 M NaCl and incubated overnight at 50°C. On the next day, the 25 hybrids are digested with RNase A (10 μ g/ml) and RNase T1 (250 U/ml) in a solution containing 10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 0.3 M NaCl for 30 min. at 25°C. Proteinase K (10 μ g) and sarcosyl (1%) were added and the incubation continued for another 30 min. at 37°C. The precipitation of RNA hybrids and resolution of 30 labeled RNAs on urea-polyacrylamide gels were done exactly as described by Peri et al. (Peri, K.G., et al., *J. Biol. Chem.*, 270: 24615-24620, 1995).

Phosphoinositide hydrolysis

The cells in 6-well dishes (5 X 10^5 /well) were 35 incubated with [3 H]-myo inositol (1 μ Ci/ml of 10

Ci/mmol specific activity: Amersham Canada, Mississauga, ON) for 24 hours in DMEM containing 5% FBS and antibiotics to label the inositol phospholipids. The cells were washed with DMEM containing 50 mM LiCl twice and incubated in the same medium for 15 min. Then the cells were stimulated with 1 μ M of PGF_{2 α} or PGE₂ for 30 min. The cells were washed with phosphate-buffered saline (PBS) once and the reactions were stopped by adding 0.4 ml of ice-cold methanol. The 5 cells were scraped, collected into 1.5 ml microfuge tubes, 0.4 ml of water and 0.4 ml of chloroform were added, vortexed vigorously for 30 sec. and centrifuged at 14,000 \times g for 10 min. The aqueous layer was applied to Dowex™ AG1-X8 (formate form) ion-exchange 10 columns (Bio-Rad, Mississauga, ON). The inositol phosphates were eluted with increasing concentrations of ammonium formate in 0.1 M formic acid exactly as 15 described by Berridge et al.

Introduction of peptides into cells

The saponin treatment of cells on ice with peptides was conducted exactly as described by Johnson et al. (Johnson, J. A., et al., *Circ. Res.*, 79: 10086-10099, 1996). Briefly, the media from cells (at 80% confluence) in 6-well dishes was removed and saved. The cells were treated with 2 ml of room temperature PBS for 2 min. followed by ice-cold PBS for an additional 2 min. on ice. The cells were then incubated for 10 min. in 2 ml of freshly prepared permeabilization buffer (20 mM HEPES, pH 7.4, 10 mM EGTA, 140 mM KCl, 50 μ g/ml saponin, 6 mM ATP and 5 mM oxalic acid) containing varying concentrations of peptides, PCP-3 and PCP-4. The cells were washed gently four times on ice with 2 ml of ice-cold PBS each time. The cells were incubated for 20 min. in the fifth wash on ice. The cells were then successively incubated for 2 min.

with 2 ml of PBS at room temperature and at 37°C. The conditioned media was returned to the cells and they were allowed to recover for 30 min. before determining phosphoinositide hydrolysis in response to prostaglandins.

Discussion

In accordance with the present invention, a novel strategy of utilizing intracellular interface between the FP receptor and the $G_{\alpha q}$ -protein as a target for designing inhibitors of FP receptor function was used. This method can be generalized to all G-protein-coupled receptors. Peptides derived from the intracellular domains of FP receptors (PCP-1 and PCP-2) and the αN and αC helices of G_q -protein (PCP-3 and PCP-4 respectively) were found to be effective inhibitors of FP receptor.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Effects of intracellular expression of PCP-1 and PCP-2 peptides on FP receptor function

Cell lines expressing the peptides, PCP-1 and PCP-2, were stimulated with 1 μM PGF_{2 α} and PGE₂ for 30 min. and the inositol phosphates were measured by anion exchange chromatography as described by Berridge et al. (Berridge, M.J., et al., *Biochem. J.*, **212**: 473-482, 1983). Briefly, the medium was discarded and the inositol triphosphate (IP3) synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation at 6000 x g for 10

min. The aqueous phase was applied to AG-1X-8 (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the 5 inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III™), the eluates were counted by scintillation spectrophotometry.

As shown in Fig. 1A, expression of either PCP-1 or PCP-2 inhibited ligand-stimulated phosphoinositide hydrolysis (n=3). Both PCP-1 and PCP-2 were stably expressed intracellularly. The cells were labeled with ³H-myoinositol for 24 hours and stimulated with 1 μM of PGF_{2α} or PGE₂ for 30 min. Inositol phosphates were separated by ion exchange chromatography and determined by scintillation counting. Data are expressed as fold stimulation in phosphoinositide hydrolysis over unstimulated controls. On the other hand, stimulation 15 of a related prostaglandin receptor expressed in these cells (with which FP receptor shows highest sequence identity among all G-protein-coupled receptors), EP1, with PGE₂, did not affect inositol phosphate generation by this receptor (Fig. 1B). Both EP1 and FP receptors 20 are coupled to Gq-class of G-proteins and generate inositol phosphates upon stimulation with ligands. The inhibition of FP receptor by ectopically expressed PCP-1 and PCP-2 peptides is specific and these peptides 25 will be modified to produce smaller and more diffusible 30 inhibitors of FP function.

EXAMPLE II

Effects of PCP-3 and PCP-4 peptides of human Gq protein on FP receptor function

The second component of interaction between the 35 FP receptor and G-protein is the domain of Gq which is

composed of α N and α C helices (Lambright, D.G., et al., *Nature*, 379: 311-319, 1996). Peptides CLSEEEAKEAR RINDEIERQL RRDKRDARRE-NH₂ (SEQ ID NO:9) (PCP-3) and KDTILQLNLK EYNLV-NH₂ (SEQ ID NO:10) (PCP-4), corresponding to α N and α C helices, respectively, were chemically synthesized using F moc chemistry and introduced transiently into permeabilized A549 cells. The cells were stimulated with PGF_{2 α} , as described above and inositol phosphate synthesis was measured. The results are expressed as fold stimulation of phosphoinositide hydrolysis by the ligand (n=3). Both α N and α C helical peptides of Gq protein inhibited agonist-induced activation of FP receptor, whereas a control peptide (poly aspartic acid, Asp6) did not affect the receptor function (Fig. 2A). PCP-3 and PCP-4, at 100 μ M, were introduced into ³H-myoinositol-labeled permeabilized A549 cells and stimulated with 1 μ M PGF_{2 α} for 30 min. Inositol phosphates were separated by ion exchange chromatography and determined by scintillation counting. A dose-response of α C peptide on FP receptor revealed a half maximal inhibitory concentration of 50 μ M of peptide under these conditions (Fig. 2B). In Fig. 2B, data are expressed as fold-stimulation by PGF_{2 α} over control cells not treated with peptide.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to

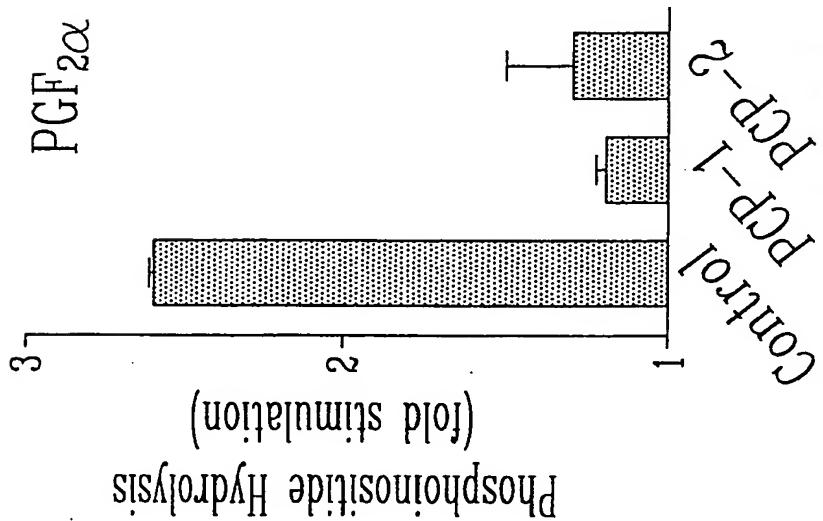
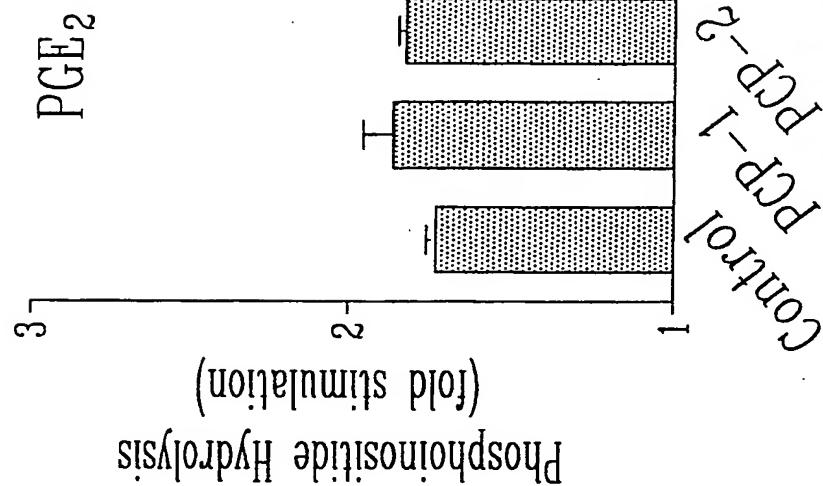
the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

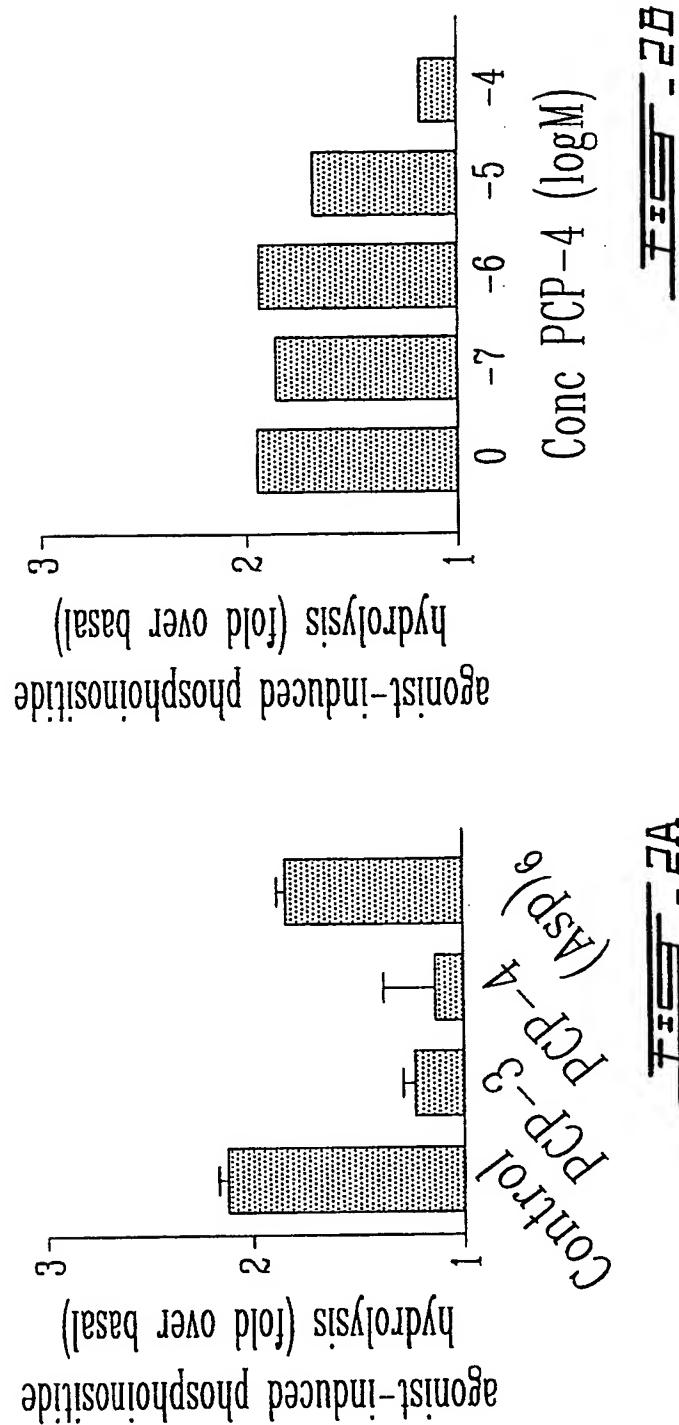
1. A receptor prostanoid receptor antagonist which binds to an intracellular molecular interface between a receptor and a G_α-protein, wherein said antagonist has a first amino acid sequence coding for a third or fourth intracellular domain, or a part thereof, and a second amino acid sequence coding for α-helices of a G_α protein, whereby when bound to the intracellular molecular interface, said antagonist hampers signal transduction from said receptor.
2. The antagonist of claim 1, wherein the receptor is the PGF_{2α} receptor of prostaglandin.
3. The antagonist of claim 2 which comprises an amino acid sequence of the FP receptor selected from the group consisting of RVKFKSQQHR QGRSHHLEM (SEQ ID NO:3) and RKAVLKNLYK LASQCCGVHV ISLHIWELSS IKNSLKVAII SESPVAEKSA ST (SEQ ID NO:4).
4. The antagonist of claim 1 which comprises an amino acid sequence derived from the sequence of at least one of the prostaglandin F_{2α} receptor and the associated protein G_{αq}.
5. A method for preventing premature delivery of fetus comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G-protein-coupled receptor antagonist which binds to an intracellular molecular interface between a receptor and a G-protein, wherein said antagonist, when bound to the intracellular molecular interface, hampers the transduction of a signal, thereby reducing contractions.

6. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G-protein-coupled receptor antagonist which binds to an intracellular molecular interface between a receptor and a G-protein, wherein said antagonist, when bound to the intracellular molecular interface, hampers the transduction of a signal thereby reducing pain associated with contractions.

1/2



2/2



SEQUENCE LISTING

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CHEMTOB, Sylvain
PERI, Krishna G.
POTIER, Michel

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01138

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/62 C07K14/47 C07K14/705 A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 21925 A (AMERICAN CYANAMID COMPANY) 17 August 1995 see the whole document ---	1-6
A	CHEMICAL ABSTRACTS, vol. 125, no. 21, 18 November 1996 Columbus, Ohio, US; abstract no. 276567, M D CARRITHERS & M R LERNER: "Synthesis and characterization of bivalent peptide ligands targeted to G-protein-coupled receptors" XP002103142 & CHEM. BIOL., vol. 3, no. 7, 1996, pages 537-542, see abstract --- ---	1-6

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

19 May 1999

01/06/1999

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01138

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 05244 A (DUKE UNIVERSITY) 2 April 1992 see the whole document -----	1-6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/01138

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 5-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 98/01138

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9521925	A 17-08-1995	US 5691188 A			25-11-1997
		AU 1846995 A			29-08-1995
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